

MICROBIAL DEGRADATION OF SEVERAL ACETAMIDE, ACYLANILIDE, CARBAMATE, TOLUIDINE AND UREA PESTICIDES

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Summary—Soil enrichment was used to isolate soil microorganisms capable of degrading isopropyl carbanilate (propham), 3',4'-dichloropropionanilide (propanil), 3'-chloro-2-methyl-*p*-valerotoluidide (solan), and methyl 3,4-dichlorocarbanilate (swep) in a muck and a silty clay loam. Degradation of the pesticides in enrichment solutions, and by pure cultures of effective microbial isolates was demonstrated by the production of the corresponding aniline, chloride ion liberation and disappearance of the parent compound. Degradation products were identified by gas-liquid and thin-layer chromatography.

Organisms isolated include *Pseudomonas striata* Chester, *Achromobacter* sp., *Aspergillus ustus* (Bain) Thom and Church, *A. versicolor* (Vuill. Tirabaschi), *Fusarium oxysporum* Schlecht, *F. solani* (Martius) Appel and Wollenweber, *Penicillium chrysogenum* Thom, *P. janthinellum* Biourge, *P. rugulosum* Thom and *Trichoderma viride* Pers. Each organism demonstrated a unique substrate specificity and was capable of degrading other aniline-based pesticides of the acetamide, acylanilide, carbamate, toluidine and urea classes.

INTRODUCTION

CARBANILATES, acylanilide and phenylurea herbicides contain aniline moieties as a basic part of their chemical structure. Numerous other pesticides also contain either anilines or chemical moieties which may be degraded to aniline type residues. Considerable interest has developed toward understanding the fate and behavior of these aniline products in the environment. Carbanilate herbicides are relatively nonpersistent (2–5 weeks) in soil, whereas phenylureas are generally more persistent (2–10 months), some persisting as long as a year or more in soil. Most acylanilide herbicides appear to remain intact only briefly in soil.

Microbial degradation is an important factor affecting the persistence of these pesticides in soil. The phenylcarbamate herbicides chlorpropham (Kaufman and Kearney, 1965; Kearney and Kaufman, 1965; Clark and Wright, 1970a, b), propham (Clark and Wright, 1970a, b), and swep (Bartha and Pramer, 1969), are hydrolyzed to their corresponding alcohol and aniline. Further decomposition of both the alcohol and aniline moieties also occurs. The acylanilide herbicides propanil and solan are hydrolyzed to their corresponding anilines and acids (Bartha, 1969; Chisaka and Kearney, 1970). Dealkylation followed by hydrolysis of the urea linkage is necessary before aniline is liberated from the dialkyl-phenylurea herbicides (Geissbuhler, 1969). More recent investigations, however, indicate that methoxy-substituted phenylurea herbicides may also be degraded directly by enzymic hydrolysis to CO₂ and the corresponding aniline and alkylalkoxyamino residue (Engelhardt *et al.*, 1971).

The ultimate fate of the chloroaniline moiety in soil is only partially understood at present. Adsorption to soil particles is known to account for some of the chloroaniline (Linke and Bartha, 1970). Bartha and Pramer (1967) reported the isolation of large quantities of

3,3',4,4'-tetrachloroazobenzene (TCAB) from soil treated with excessively high rates of 3,4'-dichloropropionanilide (propanil). This product was formed by the condensation of two 3,4-dichloroaniline metabolites. Kearney *et al.* (1970) subsequently detected TCAB residues in rice fields treated at recommended propanil application rates 2 and 3 years before sampling.

Several additional condensation products including 1,3-bis(3,4-dichlorophenyl)triazene (Plimmer *et al.*, 1970), 4-(3,4-dichloroanilino)-3,3',4'-tetrachloroazobenzene (Rosen and Siewerski, 1971) and 3,3',4,4'-tetrachloroazoxybenzene (Kaufman *et al.*, 1972) have also been identified as products of 3,4-dichloroaniline metabolism. Mixed azobenzenes, or azobenzenes resulting from the condensation of two unlike chloroaniline molecules have also been reported (Bartha, 1969; Kearney *et al.*, 1969). An understanding of the formation mechanism of such products and their fate in soil is essential if one is to prevent or reduce the production of environmental pollutants. The purpose of this investigation was to observe aniline-based herbicide degradation in several soil systems and to isolate soil microorganisms active in the degradation of these compounds.

METHODS

Pesticides

Common and chemical names, and chemical purity of all pesticides used in this investigation are listed in Table 1. All of the pesticides listed are used as herbicides with the exception of chlorphenamidine which is a miticide.

TABLE 1. COMMON AND CHEMICAL NAMES, AND PURITY OF PESTICIDES USED

Common name	Chemical name	Purity %
Alachlor	2-chloro-2',6'-diethyl- <i>N</i> -(methoxymethyl)acetanilide	98
Barban	4-Chloro-2-butynyl <i>m</i> -chlorocarbonilide	92.2+
Benefin	<i>N</i> -butyl- <i>N</i> -ethyl- <i>a,a,a</i> -trifluoro-2,6-dinitro- <i>p</i> -toliudine	98+
CDAA	<i>N,N</i> -diallyl-2-chloroacetamide	95+
CDEC	2-Chloroallyl diethyldithiocarbamate	95+
Chlorbromuron	3-[4-Bromo-3-chlorophenyl]-1-methoxy-1-methylurea	98
Chloroxuron	3-[<i>p</i> -(<i>p</i> -Chlorophenoxy)phenyl]-1,1-dimethylurea	98
Chlorphenamidine	<i>N</i> -(2-methyl-4-chlorophenyl)- <i>N',N'</i> -dimethylformamidine	95+
Chlorpropham	Isopropyl <i>m</i> -chlorocarbonilide	100.0
Cycloate	<i>S</i> -ethyl <i>N</i> -ethylthiocyclohexanecarbamate	95.2
Diallate	<i>S</i> -(2,3-dichloroallyl)diisopropylthiocarbamate	95+
Dicryl	3',4'-Dichloro-2-methylacrylanilide	98.2
Diphenamid	<i>N,N</i> -dimethyl-2,2-diphenylacetamide	96.5
Diuron	3-(3,4-Dichlorophenyl)-1,1-dimethylurea	100
DMU	3-(3,4-Dichlorophenyl)-1-methylurea	100
Fenuron	1,1-Dimethyl-3-phenylurea	100
Fluometuron	1,1-Dimethyl-3-(<i>a,a,a</i> -trifluoro- <i>m</i> -tolyl) urea	98
Metobromuron	3-(<i>p</i> -Bromophenyl)-1-methoxy-1-methylurea	98.2
Neburon	1-Butyl-3-(3,4-dichlorophenyl)-1-methylurea	100
NIA-11092	3-[3-(<i>N</i> -tert-buthylcarbamyloxy)phenyl]urea	87
Nitralin	4-(Methylsulfonyl)-2,6-dinitro- <i>N,N</i> -dipropylaniline	95
Norea	3-(Hexahydro-4,7-methanoindan-5-yl)-1,1-dimethylurea	95+
Propachlor	2-Chloro- <i>N</i> -isopropylacetanilide	95.6
Propanil	3',4'-Dichloropropionanilide	99+
Propham	Isopropyl carbonilide	100.0
Solan	3'-Chloro-2-methyl- <i>p</i> -valerotoluidide	96.4
Sweep	Methyl 3,4-dichlorocarbonilide	99

Enrichment cultures

A soil-solution enrichment technique was used to examine the microbial degradation of propham, propanil, solan and swep. Five grams of soil were added to an aqueous suspension (100 ml) of the pesticide (100 parts/10⁶) in a 250 ml flask on a reciprocating shaker. Two soils were used: a Celeryville muck and a Hagerstown silty clay loam (Table 2). Duplicate flasks were used for each soil and pesticide. Flasks with sterile soil and water were used for controls.

TABLE 2. CHEMICAL AND PHYSICAL PROPERTIES OF THE SOILS USED IN ENRICHMENT STUDIES

Soil	pH	Cation exchange capacity (m-equiv./100 g)	Moisture field capacity (%)	Organic matter (%)	Clay (%)
Celeryville muck	5.0	165.3	51.2	74.9	ND*
Hagerstown silty clay loam	6.8	14.7	25.8	2.5	39.4

* ND—not determined.

Pesticide analysis

Pesticide degradation in the enrichment solutions was demonstrated by removing samples from each flask at 2-day intervals and measuring the disappearance of the parent material, the production of the corresponding aniline and the release of chloride ion. Aniline residues were measured colorimetrically using a modification of the Bratton-Marshall reaction (Pease, 1962). Residual concentrations of the parent material were determined by first submitting a sample to alkaline hydrolysis and subsequent measurement of total aniline content (microbially evolved aniline plus hydrolyzed aniline). Chloride ion determinations were made by the procedure of Iwasaki, Utsumi and Ozawa (1952).

At the conclusion of the incubation period the enrichment solutions (approx. 70 ml) were extracted twice with 50 ml of petroleum ether. The extracts were combined, dried with powdered MgSO₄, filtered, and concentrated to a volume suitable for gas-liquid and thin-layer chromatographic analysis. Thin-layer chromatograms were developed on silica gel HF₂₅₄ in hexane:benzene:acetone (7:3:1). Gas chromatographic analyses were performed on an F and M Model 700 gas chromatograph with a flame ionization detector and a 180 cm stainless steel column packed with 10 per cent methylvinyl silicone gum rubber on diatoport S 80-100 mesh. The carrier gas (N₂) flow rate was 40 ml/min. Injection port and detector temperatures were 270 and 310°C, respectively. A column temperature of 180°C was used for detecting propham, propanil, solan and swep, and their corresponding anilines. A column temperature of 250°C was used for detecting azobenzene type compounds.

Pure cultures

Pure cultures of effective microorganisms were isolated from the enriched soils by a soil dilution plate method. Serial dilutions were prepared from the enriched soils and a set of 5 plates was prepared from each dilution. The plating medium contained: K₂HPO₄, 0.8 g; KH₂PO₄, 0.2 g; MgSO₄·7H₂O, 0.2 g; CaSO₄, 0.1 g; (NH₄)₆Mo₇O₂₄·4H₂O, 1 mg (NH₄)₂SO₄, 5.0 g; bacto-agar, 20 g; and distilled water 1000 ml. The corresponding herbicide was supplied (100 parts/10⁶) as essentially the sole source of carbon. The chemicals were introduced in 0.1 ml acetone to the sterilized and cooled (50°C) medium. One ml of

the serial dilution was added to each plate. The agar medium containing the pesticide was added to each plate, and the plates swirled to assure even distribution of the organisms throughout the plate. Plates prepared in this manner were incubated for 1–3 weeks at 24°C. Microbial cultures appearing during the incubation period were isolated, purified and maintained on the basal medium described above plus 100 parts/10⁶ of the herbicide and 0.1 g/l. of yeast extract as carbon sources. All effective cultures were identified at least to genus.

Pesticide degradation by pure cultures of microorganisms

The organisms isolated were examined for their ability to degrade chemicals analogous to the one used for their isolation. The medium used for these experiments contained the basal salts listed above in addition to 0.1 g of yeast extract and a 100 parts/10⁶ concentration of the pesticide. The pesticides were aseptically introduced in 0.1 ml acetone to each 100 ml of sterile media. One ml of actively growing cell suspensions was used as the inoculum. Incubation was at 24°C on a rotary shaker. Samples were removed daily and analyzed as described previously. Duplicate flasks were used for each experimental parameter. Controls, with and without pesticides or microorganisms were included. At the conclusion of the incubation period, the contents of one flask were extracted twice with petroleum ether and the extract analyzed as described previously. The second flask was used in an oat bioassay method to determine whether the isolated microorganisms reduced the pesticides to non-phytotoxic or less phytotoxic compounds. The addition of 100 ml of the incubated culture solution to 300 g per pot of the Hagerstown soil was equivalent to an initial application rate of 71.7 kg/ha. Inoculated pesticide solutions were compared with sterile pesticide solutions. The treated soils were planted with oat (*Avena sativa* L. var. Markton) seeds. The seedlings were harvested after a 3-week growing period, and the fresh weight was expressed as percentage of the sterile control with no pesticide.

RESULTS AND DISCUSSION

Soil enrichment

Microbial degradation of propham, propanil, solan, and swep was observed in soil enrichment cultures (Figs. 1–3). Lag periods of only 2–5 days were observed for the various chemicals. No degradation was observed in similar sterilized culture systems. The rate of microbial degradation of the pesticides varied with the pesticides, soil type and in the case of propham, with the replication. Considerable variation was observed between replications and in experiments in the extent of propham biodegradation (Fig. 1). Several patterns of propham biodegradation were observed. Propham degradation occurred more readily and more completely in enrichment cultures containing muck soil. In some instances propham degradation occurred readily with no detectable accumulation of aniline, whereas in others aniline accumulated before it was degraded. Carbanilate pesticides and their corresponding aniline and alcohol are hydrolyzed to CO₂ by soil microorganisms (Kaufman and Kearney, 1965; Kearney and Kaufman, 1965; Clark and Wright, 1970a, b). The variation in experimental results observed with propham could be characteristic of the population developing during the enrichment period. Populations capable of rapidly metabolizing aniline may have developed in some enrichment solutions but not in others. Alternatively, microbial populations capable of degrading propham by mechanisms not involving aniline liberation could have been present in some enrichment cultures. Other experiments we have conducted which will be reported subsequently, indicate some soil microorganisms may hydrolyze propham to aniline, isopropyl alcohol and CO₂, and subsequently use the aliphatic moiety

as a carbon source and ignore the aromatic moiety. A predominance of this type of organism in the enrichment flora would result in the accumulation of aniline which may then be degraded by a subsequent enrichment flora of its own. No attempt was made to characterize such microbial population differences in these enrichment cultures.

Propanil and swep degradation resulted in the liberation of 3,4-dichloroaniline. Propanil was readily degraded in both soil types, whereas swep degradation occurred only in enrichment cultures containing muck. Further degradation of the 3,4-dichloroaniline moiety was not observed. Trace amounts (1 per cent) of the 3,3',4,4'-tetrachloroazobenzene

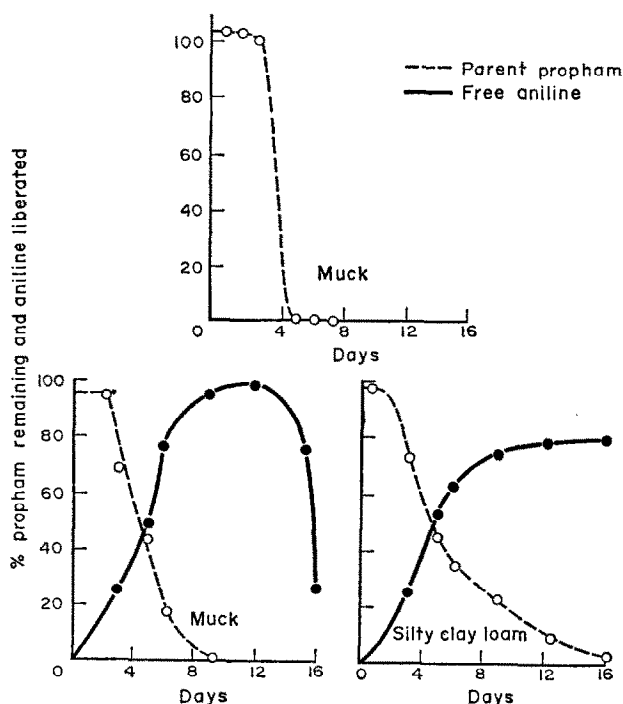


FIG. 1. Patterns of prophan biodegradation observed in soil enrichment cultures.

(TCAB) were observed in ether extracts of the enrichment solution at the conclusion of the incubation period. Identity of TCAB was established through gas-liquid and thin-layer chromatographic comparisons with a known standard.

Solan degradation occurred in both muck and silty clay loam enrichment solutions. 3-Chloro-*p*-toluidine was a product which accumulated during the enrichment period, but was subsequently degraded in enrichment cultures containing muck. Degradation of the toluidine moiety was accompanied by a nearly quantitative release of the chloride ion. No azobenzene formation was observed in solan enrichment cultures.

Isolation of pesticide degrading microorganisms

Numerous soil microorganisms were isolated from the various enrichment cultures. In several instances similar organisms were isolated on different compounds. Morphological comparisons and metabolic characterizations of these organisms with several analogous pesticides indicated that differences between these similar species were relatively minor.

Therefore, only single cultures were maintained for further study. Soil fungi isolated from the enrichment cultures included *Aspergillus ustus* (Bain) Thom and Church, *A. versicolor* (Vuill. Tirabaschi), *Fusarium oxysporum* Schlecht, *F. solani* (Martius) Appel and Wollenweber, *Penicillium chrysogenum* Thom, *P. janthinellum* Biourge, *P. nigulosum* Thom and *Trichoderma viride* Pers. Only two distinctly different bacterial strains were isolated: *Pseudomonas striata* Chester and *Achromobacter* sp. The source of these cultures is shown in Table 3.

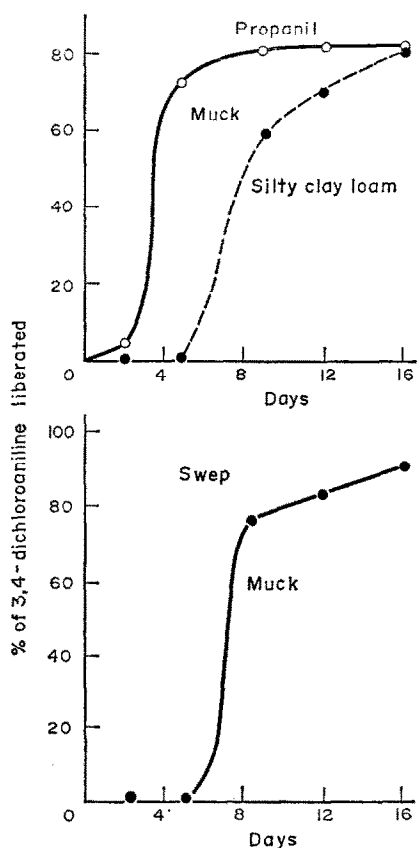


FIG. 2. Liberation of 3,4-dichloroaniline from propanil and sweep in soil enrichment cultures.

Soil fungi were predominately isolated on solan and propanil whereas bacteria were more common in propham and sweep enrichment cultures. *Fusarium oxysporum* Schlecht was the most prevalent soil fungus isolated, whereas *Pseudomonas striata* Chester was the most prevalent bacterium. The ability of these organisms to metabolize other aniline-based, acetamide, or thio- and dithiocarbamate herbicides was determined under pure culture conditions.

Substrate specificity of microbial isolates

All of the microbial isolates obtained from the enrichment cultures were examined for their ability to degrade other phenylamide and carbamate pesticides. Differences were observed in the abilities of all soil fungus isolates to metabolize chlorphenamidine, chlorpro-

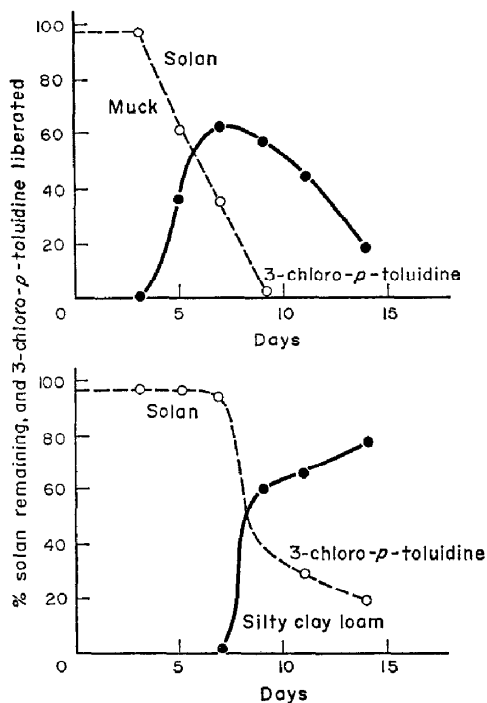


FIG. 3. Microbial degradation of solan in soil enrichment cultures.

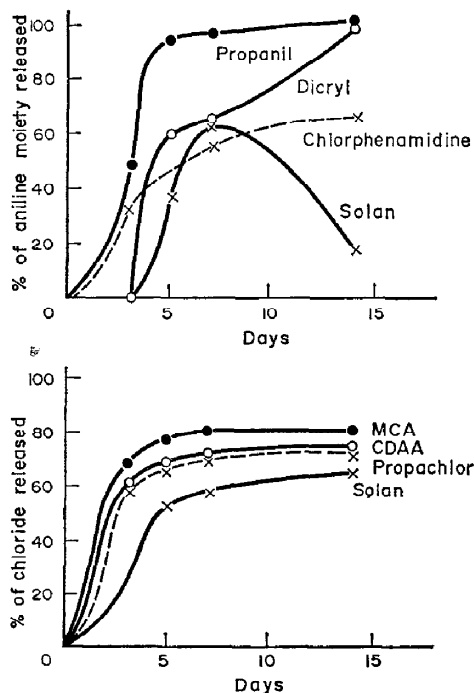


FIG. 4. Liberation of chloroanilines and chloride ion from several pesticides by *F. oxysporum*. (MCA = monochloroacetate.)

pham, dicryl, diuron, propanil, propachlor, propham and solan (Table 4). Each of the fungus isolates appeared unique in their substrate specificity. All of the fungi metabolized several aniline-based herbicides and liberated the corresponding aniline, or Cl^- . Each appeared unable to metabolize one or more of the pesticides. Several organisms appeared to liberate Cl^- without the liberation of detectable amounts of the corresponding aniline. Whether this was a result of rapid utilization of the liberated aniline or a dehalogenation without hydrolysis of the phenylamide linkage was not determined. Subsequent investigations (Kaufman *et al.*, 1971) with propachlor indicate that dehalogenation to hydroxypropachlor is a mechanism of microbial degradation for this molecule. Thus the possibility for dehalogenation without hydrolysis of the phenylamide linkage cannot be discounted.

Fusarium oxysporum was selected for further investigation to determine its ability to metabolize a more inclusive group of aniline-based or carbamate pesticides (Table 5). This organism possessed the ability to degrade a large number of carbanilate, acylanilide,

TABLE 3. SOIL MICROORGANISMS ISOLATED FROM SOIL ENRICHMENT CULTURES CONTAINING PESTICIDES

Organism	Enrichment cultures from which organisms were obtained			
	Propham	Propanil	Solan	Sweep
<i>Aspergillus ustus</i>		×		
<i>A. versicolor</i>		×	×	
<i>Fusarium oxysporum</i>	×	×	×	×
<i>F. solani</i>		×	×	
<i>Penicillium chrysogenum</i>	×	×	×	
<i>P. janthinellum</i>	×	×	×	
<i>P. rugulosum</i>		×	×	
<i>Trichoderma viride</i>	×	×	×	
<i>Pseudomonas striata</i>	×	×	×	×
<i>Achromobacter</i> sp.	×		×	

phenylurea, acetamide and dithiocarbamate pesticides. This metabolism led to some detoxication of the pesticides when oat seedlings were used as a bioassay plant for culture solutions added to soil. No attempt was made in this investigation to establish identity of all metabolic products detected on t.l.c. plates. The corresponding anilines were detected from most aniline-based pesticides. The monoalkylated derivative (DMU) of diuron was detected in culture solutions initially containing diuron.

Extensive release of Cl^- occurred during the metabolism of solan, CDAA and propachlor by *F. oxysporum* (Tables 4-5, Fig. 4). 3-Chloro-*p*-toluidine was positively identified as a product of solan metabolism through co-chromatography in both gas-liquid and t.l.c. systems. This moiety was also rapidly degraded with the liberation of Cl^- (Fig. 4). The degradation of propachlor by *F. oxysporum* has been examined in detail and reported elsewhere (Kaufman *et al.*, 1971). The major route of degradation involved dehalogenation and the formation of hydroxypropachlor, thus hydrolysis of the amide linkage was not necessary for degradation to occur. Hydrolysis of the amide linkage of CDAA and propachlor would result in the formation of monochloroacetate (MCA) and diallylamine or isopropylaniline, respectively. *F. oxysporum* was capable of dehalogenating MCA (Fig. 4). Whether dehalogenation of CDAA occurred with or without hydrolysis of the amide

TABLE 4. EFFECT OF SEVERAL SOIL FUNGI ON SELECTED ANILINE-BASED PESTICIDES

Organism	Substrate	Aniline moiety (%) detected on day			Chloride ion (%) detected on day 7
		3	5	7	
<i>Aspergillus ustus</i>	Chlorphenamidine	0	2.8	19.5	4.7
	Chlorpropham	0	0	0	5.7
	Dicryl	0	5.0	13.5	13.0
	Diuron	0	0	0	3.0
	Propanil	1.3	6.1	33.0	12.9
	Propachlor	0	0	0	22.2
	Propham	0	0	0	0
	Solan	0	0	0	0
<i>Aspergillus versicolor</i>	Chlorphenamidine	0	12.5	29.9	36.9
	Chlorpropham	0	0	0	0
	Dicryl	1.5	2.1	4.3	13.4
	Diuron	0	0	0	0
	Propanil	5.4	14.8	16.2	4.9
	Propachlor	0	0	0	0
	Propham	0	0	1.0	0
	Solan	1.7	5.9	11.9	72.6
<i>Fusarium oxysporum</i>	Chlorphenamidine	0	5.6	29.2	3.1
	Chlorpropham	0	0	0	39.2
	Dicryl	0	9.2	7.1	5.7
	Diuron	0	0	0	0
	Propanil	45.1	56.5	49.8	4.5
	Propachlor	0	0	0	29.9
	Propham	0	0	1.0	0
	Solan	0	18.6	25.4	5.7
<i>Fusarium solan</i>	Chlorphenamidine	0.7	1.4	1.7	13.9
	Chlorpropham	0	0	0	0
	Dicryl	9.9	30.5	32.7	6.1
	Diuron	0	0	0	0
	Propanil	8.8	36.0	38.7	0
	Propachlor	0	0	0	100.0
	Propham	0	0	2.4	0
	Solan	2.1	22.0	10.2	25.3
<i>Penicillium chrysogenum</i>	Chlorphenamidine	0	0	0	0
	Chlorpropham	0	0	0	0
	Dicryl	3.6	48.3	87.4	8.1
	Diuron	0	0	0	0
	Propanil	10.1	29.6	71.3	4.0
	Propachlor	0	0	0	0
	Propham	0	19.3	14.5	0
	Solan	49.2	57.6	60.2	11.8
<i>Pencillium janthinellum</i>	Chlorphenamidine	0	0	4.9	0
	Chlorpropham	1.7	0	0.8	0
	Dicryl	10.7	26.9	60.4	1.1
	Diuron	0	0	0	0
	Propanil	14.1	19.5	30.3	0
	Propachlor	0	0	0	5.8
	Propham	36.6	38.5	14.5	0
	Solan	0	25.4	29.7	1.7

TABLE 4—continued

Organism	Substrate	Aniline moiety (%) detected on day			Chloride ion (%) detected on day 7
		3	5	7	
<i>Pencillium rugulosum</i>	Chlorphenamidine	0	0	6.0	3.3
	Chlorpropham	0	0	0	0
	Dicryl	0	2.1	9.2	0.2
	Diuron	0	0	0	0
	Propanil	1.4	0	7.4	0
	Propachlor	0	0	0	0
	Propham	4.8	0	0	0
	Solan	2.6	25.4	41.5	0.3
<i>Trichoderma viride</i>	Chlorphenamidine	0	0	2.1	0
	Chlorpropham	0	0	0	0
	Dicryl	1.8	21.0	54.0	19.7
	Diuron	0	0	0	0
	Propanil	1.4	19.5	77.4	4.7
	Propachlor	0	0	0	34.5
	Propham	0	0	1.0	0
	Solan	0	6.4	19.9	8.8

TABLE 5. DEGRADATION OF PESTICIDES BY *F. oxysporum* IN 20-DAY INCUBATION PERIOD

Pesticide	Aniline moiety % detected at 20 days	Halide % detected at 20 days	Oat seedling bioassay, fresh weight: control %	Products detected on t.l.c. plates
Alachlor	0	22.0	ND*	+
Barban	0.5	31.8	37.0	+
Benefin	0	ND	100.0	—
CDAA	ND	100.0	96.3	+
CDEC	ND	23.6	14.8	—
Chlorobromuron	1.8	9.5	7.4	+
Chloroxuron	0	30.7	63.0	+
Chlorphenamidine	11.8	13.9	88.9	+
Chlorpropham	0	11.0	0	+
Cycloate	0	ND	0	—
Diallate	ND	90.3	0	—
Dicryl	19.2	8.1	92.6	+
Diphenamid	ND	ND	0	—
Diuron	0	0	7.4	+
DMU	0.3	69.4	25.9	+
Fenuron	0	ND	11.1	+
Fluometuron	0	ND	7.4	—
Metobromuron	3.0	12.2	7.4	+
Neburon	0	26.7	96.3	+
NIA-11092	1.3	ND	7.4	+
Nitralin	0	ND	59.3	+
Norea	9.7	ND	14.8	+
Propachlor	0	100.0	88.9	+
Propanil	34.3	23.0	77.8	+
Propham	7.2	ND	0	+
Solan	0.9	100.0	100.0	+
Swept	0.7	5.8	22.2	+

* ND—Not determined.

linkage was not determined in the present investigation. Based on the results of this investigation and others (Kaufman *et al.*, 1971) it seems probable that dehalogenation of CDAA or its products could occur either before or after hydrolysis of the amide linkage.

Both bacterial isolates were examined for their ability to degrade other phenylamide, acetamide and carbamate herbicides. The two organisms were similar in their substrate specificity. Both were most active on chlorpropham and propham and only slightly active on phenylureas (diuron, DMU, fenuron). Oat seedling bioassay of culture solutions of *P. striata* originally containing chlorpropham, dicryl, propham and solan indicated not only

TABLE 6. DEGRADATION OF HERBICIDES BY TWO BACTERIAL ISOLATES IN 6-DAY INCUBATION PERIOD

Herbicide	Organism	Aniline moiety % detected at 6 days	Halide ion % detected at 6 days	Oat seedling bioassay, fresh weight: control %
CDAA	1*	ND†	36.8	0
	2	ND	24.5	0
CDEC	1	ND	7.9	42.9
	2	ND	31.5	56.3
Chlorpropham	1	0	100.0	114.3
	2	0	100.1	93.8
Cycloate	1	0	ND	0
	2	0	ND	0
Dicryl	1	67.5	8.1	121.4
	2	56.8	12.1	93.8
Diuron	1	0	0	7.1
	2	0	4.1	3.1
DMU	1	0	0	6.3
	2	0	3.9	0
Fenuron	1	0	ND	7.1
	2	0	ND	6.3
Propachlor	1	0	0	67.9
	2	0	15.3	75.0
Propanil	1	55.2	0	89.3
	2	58.6	0	81.3
Propham	1	0	ND	107.1
	2	0	ND	100.0
Solan	1	0	67.6	110.7
	2	0	84.5	93.8
Swept	1	6.1	7.7	96.4
	2	8.8	31.0	93.8

* Organism 1 = *P. striata*; 2 = *Achromobacter* sp.

† ND—Not determined.

that detoxication had occurred, but that some growth promoting substances may have been produced. Although the exact nature of this growth stimulation was not characterized, the influence of various herbicides on production of growth-regulator type substances has been reported (Sobieszczanski, 1970).

The results of this investigation indicate that numerous acetamide, acylanilide, carbamate, toluidine and urea pesticides are biodegraded by a variety of soil microorganisms. Each of the soil microorganisms isolated demonstrated a unique range of substrate specificity, but all were capable of degrading and dehalogenating a variety of pesticides. Such results illustrate the adaptability and omnivorous nature of soil microbial populations with respect to certain chemical classes of pesticides.

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